Preparation of Protected Ribonucleosides Suitable for Chemical Oligoribonucleotide Synthesis

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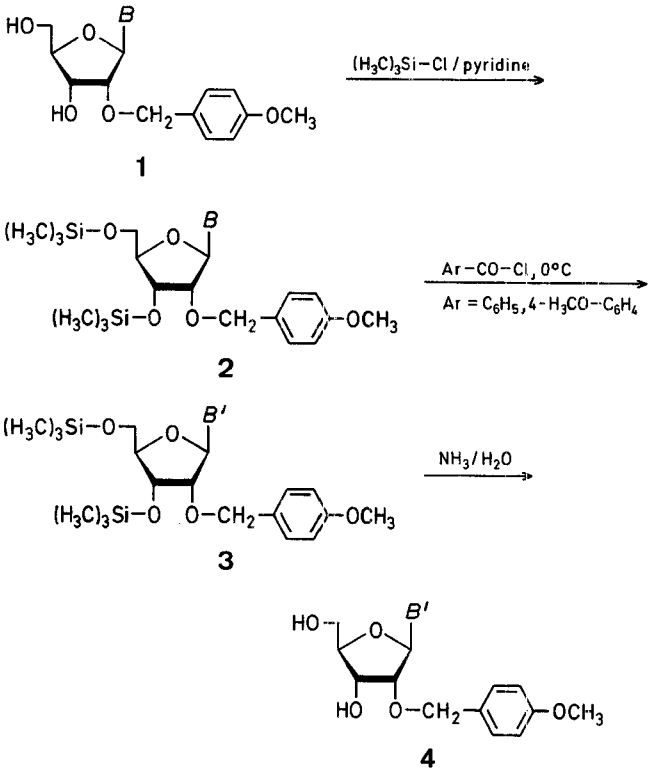
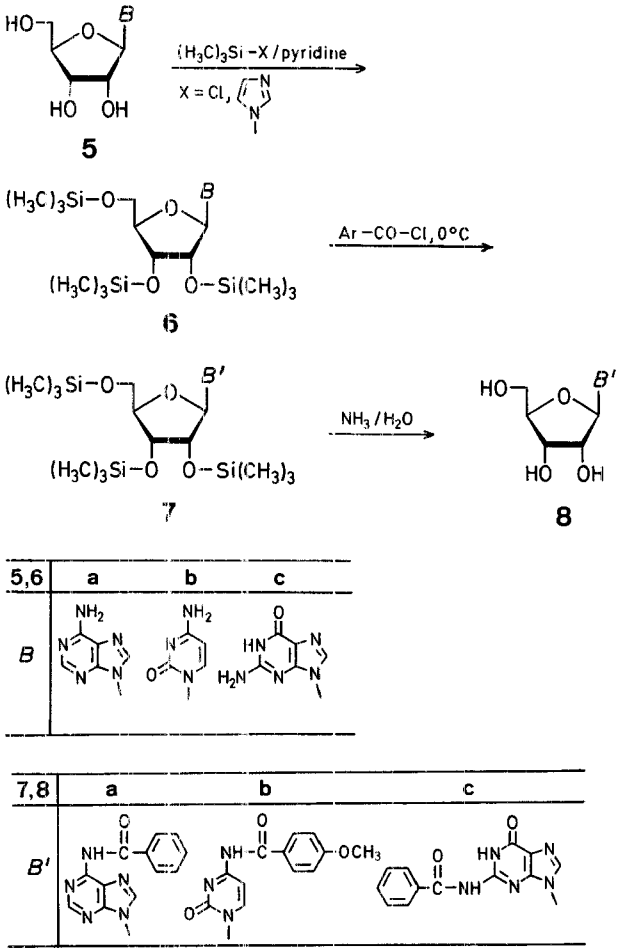
The synthesis of oligoribonucleotides has lagged behind recent advances in corresponding oligodeoxyribonucleotide syntheses largely due to the difficulty and time required to prepare the desired protected ribonucleoside building blocks. Recently, the use of *p*-methoxybenzyl bromide for direct protection of the 2'-hydroxy group of the four common ribonucleosides has been described[1,2]. This paper reports that by using such 2'-protected derivatives the nucleobase can be directly protected in a single step which involves transient protection of the remaining hydroxy groups as the trimethylsilyl ether derivatives. This results in a two step preparation of the desired nucleobase protected and 2'-hydroxy protected ribonucleoside derivatives suitable for oligoribonucleotide synthesis. Additionally, the procedure can be used to prepare in a single step the nucleobase protected ribonucleoside derivatives in an analogous fashion to that which has been described for 2'-deoxyribonuclosides[3,4].

The 2'-substituted derivative of adenosine **1a** and cytidine **1b**, were prepared as described[1,2]. The isolated products were analyzed by N.M.R. and isomer purity was determined by high performance liquid chromatography. Subsequent reaction with trimethylsilyl chloride or trimethylsilylimidazole produced quantitatively, as determined by T.L.C. analysis (dichloromethane/methanol, 9/1), the intermediate compounds **2a** and **2b**. Subsequent addition of benzoyl chloride or anisoyl chloride and hydrolysis of the trimethylsilyl ethers during work-up produced the desired products **4a, b** in high yield.

Direct protection of the nucleobase moiety of cytidine, adenosine and guanosine also occurs with high yields with this procedure (Scheme **B**). In the first step the 2',3',5'-*O*-tris[trimethylsilyl] ether derivatives **6** were prepared. The ether derivatives have reasonable stability and can in the case of guanosine be isolated in analytically pure form (**6c**). In all three cases the reaction was quantitative as determined from T.L.C. analysis, however, owing to the high solubility we were unable to crystallize significant amounts of the cytidine or adenosine derivative. An analytically pure sample of the guanosine derivative **6c** could be obtained but the moderate yield (54%) was also related to its relatively high solubility.



Scheme A



Scheme **B**

In all cases described here compounds of exceptional purity were obtained. The sample of *N*-benzoyl-2'-*O*-*p*-methoxybenzyladenosine (**4a**) melted very sharply at 150°C. H.P.L.C. analysis of the starting 2'-*O*-*p*-methoxybenzyladenosine indicated a 2'- to 3'-isomer ratio of 99.8 to 0.2. We are at present unable to account for the difference in melting points between the sample prepared by the present procedure and that previously reported[1].

The present procedure combined with direct protection of the 2'-hydroxy group[1,2] allows rapid preparation of key intermediates necessary for oligonucleotide synthesis[5,6,7].

Table. *N*-Aroylribonucleosides **8a–c** prepared

8	Yield [%]	m.p. [°C] found	m.p. [°C] reported	Molecular formula[a]	^{1}H-N.M.R. (DMSO-d_6)[b] δ [ppm]
a	88	133–134°	134–135°[9]	$C_{17}H_{17}N_5O_5$ (371.4)	3.64 (s, HOD); 4.01 (m, 1H, CH); 4.20 (m, 1H, CH); 4.65 (m, 1H, CH); 6.06 (d, 1H, J = 5.9 Hz, H-1'); 7.60 (m, 3H, ArH); 8.03 (m, 2H, ArH); 8.72 (s, 1H, H-2); 8.77 (s, 1H, H-8)
b	84	185–187°	178–179°[8]	$C_{18}H_{19}N_3O_7$ (389.4)	3.62 (s, HOD); 3.82 (s, 3H, OCH_3); 3.7–4.1 (m, 3H, CH); 5.82 (d, 1H, J = 2.6 Hz, H-1'); 7.05 (d, 2H, J = 8.9 Hz, ArH); 7.33 (d, 1H, J = 7.5 Hz, H-5); 8.00 (d, 2H, J = 8.8 Hz, ArH); 8.46 (d, 1H, J = 7.5 Hz, H-6)
c	78	254–255°	240–241°[10]	$C_{17}H_{17}N_5O_6$ (387.4)	3.58 (s, HOD); 3.93 (m, 1H, CH); 4.15 (m, 1H, CH); 4.49 (m, 1H, CH); 5.90 (d, 1H, J = 6.0 Hz, H-1'); 7.68 (m, 3H, ArH); 8.04 (m, 2H, ArH); 8.29 (s, 1H, H-8)

[a] Satisfactory microanalysis obtained: C ±0.25; H ±0.13; N ±0.12.
[b] Recorded in the presence of a small amount of D_2O; chemical shifts are given with respect to TMS (0.00 ppm).

N-Benzoyl-2'-*O*-*p*-methoxybenzyladenosine (4a) or N-Anisoyl-2'-*O*-*p*-methoxybenzylcytidine (4b):

To 2'-*O*-*p*-methoxybenzyladenosine[1] (**1a**; 3.49 g, 9 mmol) or 2'-*O*-*p*-methoxybenzylcytidine hydrochloride[2] (**1b**; 3.60 g, 9 mmol) in dry pyridine (70 ml) is added trimethylsilyl chloride (4.45 g, 41 mmol). After stirring 2 h at ambient temperature the mixture is cooled to 0 °C and benzoyl chloride (2.53 g, 18 mmol) or anisoyl chloride (3.07 g, 18 mmol) is added dropwise over a period of 30 min. The mixture is allowed to warm slowly to 25 °C and stirred an additional 2 h. The reaction is stopped by addition of water (10 ml) at 0 °C with stirring. After stirring 5 min at 0 °C and a further 5 min at 25 °C, concentrated aqueous ammonia (20 ml) is added. After stirring an additional 15 min the mixture is partitioned between equal volumes (100 ml) of dichloromethane and 1 molar triethylammonium hydrogen carbonate (pH 7.5). The dichloromethane phase was washed with water (2 × 40 ml), dried with magnesium sulfate and evaporated to dryness. The resulting foam is crystallized from absolute ethanol (adenosine derivative) or ethyl acetate (cytidine derivative).

N-Benzoyl-2'-O-p-methoxybenzyladenosine (**4a**); yield: 89%; m.p. 150–151 °C (Lit.[1] m.p. 186–187°)

$C_{25}H_{25}N_5O_6$	calc.	C 61.10	H 5.14	N 14.26
(491.5)	found	61.04	5.23	14.11

^{1}H-N.M.R. (DMSO-d_6): δ = 3.58 (s, HOD); 3.68 (s, 3H, OCH_3); 4.06 (q, 1H, J = 3.1 Hz, CH); 4.36–4.66 (m, 4H, CH, $ArCH_2$); 6.16 (d, 1H, J = 6 Hz, H-1'); 6.73 (d, 2H, J = 8.6 Hz, ArH); 7.07 (d, 2H, J = 8.6 Hz, ArH); 7.60 (m, 3H, ArH); 8.04 (m, 2H, ArH); 8.64 (s, 1H, H-2); 8.69 ppm (s, 1H, H-8).

N-Anisoyl-2'-O-p-methoxybenzylcytidine (**4b**); yield: 80%; m.p. 178–180 °C.

$C_{25}H_{27}O_8N_3$	calc.	C 60.36	H 5.43	N 8.45
(497.6)	found	60.20	5.41	8.53

^{1}H-N.M.R. (DMSO-d_6): δ = 3.59 (HOD); 3.71 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.65–4.11 (m, 5H, CH, CH_2); 4.68 (s, 2H, $ArCH_2$); 5.97 (d, 1H, J = 2.7 Hz, H-1'); 6.87 (d, 2H, J = 8.6 Hz, ArH); 7.05 (d, 2H, J = 8.9 Hz, ArH); 7.30 (m, 3H, ArH, H-5); 8.00 (d, 2H, J = 8.9 Hz, ArH); 8.42 ppm (d, 2H, J = 7.6 Hz, H-6).

N-Aroylribonucleosides 8a–c; General Procedure:

To a suspension of the ribonucleoside **5a–c** (30 mmol) in dry pyridine (225 ml) is added trimethylsilyl chloride (24.44 g, 225 mmol). After stirring 2 h at ambient temperature the mixture is cooled to 0 °C and benzoyl chloride (12.65 g, 90 mmol) or anisoyl chloride (15.35 g, 90 mmol) is added dropwise over a period of 30 min. The mixture is allowed to warm to 25 °C and stirred for additional 2 h. The reaction is stopped by the addition of water (30 ml) at 0 °C and stirred a further 5 min at 25 °C then concentrated aqueous ammonia (60 ml) is added. After stirring an additional 15 min the mixture is poured into water (500 ml) and extracted with dichloromethane (100 ml). The water phase is evaporated until crystallization is initiated. Recrystallization of the product is from water. See Table for yields and physical data.

2',3',5'-*O*-Tris[trimethylsilyl]guanosine (6c):

To a suspension of guanosine (**5c**; 0.71 g, 2.5 mmol) in dry tetrahydrofuran (30 ml), at −10 °C is added trimethylsilylimidazole (2.10 g, 15 mmol). After stirring 1.5 h T.L.C. analysis indicates complete conversion to a compound with a higher R_f value. The mixture is partitioned between dichloromethane (50 ml) and water (50 ml), dried with sodium sulfate, and the solvent removed. The residue is crystallized from tetrahydrofuran (20 ml) after cooling to −20 °C; yield: 0.68 g (54%); m.p. 220–222 °C.

$C_{19}H_{37}O_5N_5Si_3$	calc.	C 45.65	H 7.41	N 14.01
(499.8)	found	45.80	7.46	14.21

^{1}H-N.M.R. ($CDCl_3$): δ = 0.10 (s, 9H, CH_3); 0.17 (s, 9H, CH_3); 0.20 (s, 9H, CH_3); 1.81 (s, 2H, NH_2); 3.72–3.98 (m, 2H, CH_2); 4.12 (m, 1H, CH); 4.29 (m, 1H, CH); 4.45 (m, 1H, CH); 5.86 (d, 1H, J = 3.8 Hz, H-1'); 6.16 (s, 1H, NH); 7.97 ppm (s, 1H, H-8).

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